UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/820,975	04/07/2004	Daniel Santi	36707.00018/010110.00	9532

81228 7590 07/20/2009

Fox Rothschild LLP
Bristol-Myers Squibb
2000 Market Street
10th Floor
Philadelphia, PA 19103

EXAMINER	
POPA, ILEANA	

ART UNIT	PAPER NUMBER
1633	

MAIL DATE	DELIVERY MODE
07/20/2009	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/820,975	SANTI ET AL.	
	Examiner	Art Unit	
	ILEANA POPA	1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *21 April 2009*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-19 and 31-39* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-19 and 31-39* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

1. Claims 20-30 have been cancelled. Claims 2, 6,14, 19, 32, 36, 37, and 39 have been amended.

Claims 1-19 and 31-39 are pending and under examination.

Response to Arguments

Claim Rejections - 35 USC § 103

2. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

3. Claims 1-19 and 31-39 remain rejected under 35 U.S.C. 103(a) as being unpatentable over Hodgson (PGPUB 2002/0025561), in view of each Padgett et al. (Gene, 1996, 168: 31-35), Resnick et al. (WO 98/01573), and Gokhale et al. (Science, 1999, 284: 482485).

Hodgson teaches a method of obtaining a synthetic gene by ligating three DNA segments, the method comprising: **(a)** providing three different DNA vectors each comprising a selectable marker and a different DNA insert, wherein all DNA inserts are flanked by identical type IIS restriction sites, **(b)** cleaving each DNA vector with a type IIS enzyme to generate segments with region of identity (or ligatable ends) with an

adjacent segment, **(c)** simultaneously ligating the three DNA segments, and selecting the ligation product based on the presence in the vector of the selectable marker; one or more of the DNA segments could comprise the vector and the final ligation product is a complete recombinant DNA/vector, which could be made either linear or circular (i.e., the final ligation product comprises a selection marker from one of the three vectors), and **(d)** transforming cells with the final ligation product and selecting the transformants comprising the ligation product based on the presence of the selection marker above (claim 1) (p. 3, paragraphs 0030 and 0031, claims 1, 5, 9, and 10). It is noted that, in order to assembly the gene from the three different DNA segments, the three different segments must be assembled in the correct order, i.e., each of the end DNA segments must necessarily comprise one region of identity (ligatable end) with the internal segment; therefore, in order to be both end DNA fragments, the internal segment must necessarily comprise two regions of identity (ligatable ends). Hodgson teaches that the three inserts are flanked by identical type IIS restriction sites; therefore, the first and third cleavage sites are identical, the second and fourth cleavage sites are identical, and the 5' and 3' cleavage site in the same or two different Type 3 DNA molecules are identical (claims 2, 7-10, 14, 16-19). Hodgson also teaches and that cleavage with type IIS enzymes generates three segments with region of identity (or ligatable ends) with the adjacent segment; therefore, cleavage of the second site produces a single-stranded overhang in the in the first segment which is ligatable to a single-strand overhang of an adjacent segment, cleavage of the fourth site produces a single-stranded overhang in the in the second segment which is ligatable to a single-strand

overhang of an adjacent segment, while cleavage at the 5' and 3' sites in the third fragment produces 5' and 3' single-strand overhands which are ligatable to the single-strand overhangs of two adjacent fragments (claim 14). Additionally, Hodgson teaches that one or more of the DNA segments could comprise the vector (see above), i.e., the resulting linear first and second DNA molecule comprise the DNA segments covalently associated with the vectors having the selectable markers (claim 35).

Hodgson does not teach ligating the digested DNA segments without purifying the digested fragments (claims 1, 2, 14, and 35). However, at the time the invention was made, ligating digested fragments without purifying them was taught by the prior art; the prior art also taught that the presence of the type IIS restriction endonuclease in the ligation reaction reduces the cloning time, provides selection for the desired ligation product, and results in efficient ligation which is suitable for difficult cloning experiments (see Padgett et al., p. 34, column 1; p. 35, column 1). It would have been obvious to one of skill in the art, at the time the invention was made, to modify the Hodgson's method by eliminating the purification step after digestion, with a reasonable expectation of success. One of skill in the art would have been motivated to do so in order to simplify the procedure and select for the desired ligation product. One of skill in the art would have been expected to have a reasonable expectation of success because the prior art teaches that such a simplified ligation procedure can be successfully used to obtain the desired product.

Hodgson and Padgett et al. do not each vector as comprising a selectable and counter selectable marker, with each vector comprising a distinct set of selectable and

counter selectable markers (claims 2-10, 13-19 and 31-39). However, at the time the invention was made, the prior art taught the use of a combination of vectors each vector having a distinct set of selectable and counter selectable markers for the accurate selection of the final recombinant product comprising the desired insert, wherein the selectable marker could be the tetracycline resistance gene and the counter selectable marker could by the ccdB (see Resnick et al., p. 10, lines 9-15, p. 11, lines 24-26, p. 20, lines 9-30, p. 21, lines 15-25, p. 23, lines 23-28, p. 35, lines 20-26, p. 78, lines 1-10). One of skill in the art would have known to use the right combination of selectable and counter-selectable markers for a more efficient selection of the desired product. One of skill in the art would have also known to use selection based on the presence of both the first and the second selectable markers when cleaving the DNA vectors such as to obtain two of the DNA segments covalently attached to the vector to ensure that both segments are present in the final ligation product.

Hodgson, Padgett et al., and Resnick et al. do not teach PKS (claims 11 and 12). Gokhale et al. teach recombining modules from the naturally-occurring PKSs (p. 482, column 2). It would have been obvious to one of skill in the art, to use the method of Hodgson, Padgett et al., and Resnick et al. to obtain diverse synthetic PKSs as taught by Gokhale et al., with a reasonable expectation of success. The motivation to do so is provided by Gokhale et al. who teach PKSs have a modular structure, and novel combinations of modules could result the synthesis of diverse medicinally important new products (Abstract, p. 482, column 1). One of skill in the art would have been expected

to have a reasonable expectation of success in making such synthetic genes because Hodgson teach the successful *in vitro* synthesis of genes by his method.

It is noted that the instant specification defines Type I, II, and III vectors as vectors containing an insertion site for the DNA segment and selectable markers, wherein the only difference between the Type I, II, and III vectors is that each contains a different the selectable marker as compared to the others (see p. 19, paragraph 0230). Therefore, by using a method according to the combined teachings of Hodgson, Padgett et al., Resnick et al., and Gokhale et al. (i.e., employing different selectable and/or counter selectable markers on each vector), one of skill in the art would use Type I, II, and III molecules.

With respect to the limitation of the presence of at least two Type 3 DNA molecules (claims 15 and 18), it is noted that the Type 3 DNA molecules contain the interior segments. One of skill in the art would know to use more than one Type 3 DNA molecule, depending on the need to add more modules to the synthetic gene especially that Hodgson teaches his method as being suitable to be used with multiple internal segments (p. 3, paragraph 0031). With respect to claim 4, one of skill in the art would have been motivated to isolate the final ligation product from the transformants in order to sequence or transfer it to another vector, as needed; it is noted that such isolation is routine in the art.

Thus, the claimed invention was *prima facie* obvious at the time the invention was made.

Applicant traversed the instant rejection on the grounds that invention described allows rapid, economic and efficient preparation of artificial genes. Unlike the prior art methods described by the Examiner, the invention provides a practical method for making very large synthetic genes (e.g., including genes greater than 20 kbp in length) by a process including simultaneous ligation of multiple fragments. The claimed method makes use of a combination of three different types of vectors, and, in contrast to prior art methods, does not require multiple rounds of purification of short isolated inserts (e.g., by gel electrophoresis). Elimination of the need for isolation and recloning of small polynucleotide fragments is a significant advantage over the prior art methods in the references cited by the Examiner.

Applicant notes that, although the Examiner cited "Steward et al." along with Hodgson, Padgett et al., Resnick et al., and Gokhale et al., the citation of Steward et al. was not provided.

Applicant argues that the Examiner has failed to provide enough evidence to show that all the limitations of the rejected claims have been taught by the references cited. In making a rejection, the Examiner should not engage in a hindsight picking and choosing. Applicant argues that, to establish obviousness, it is not enough that the pieces of information about the elements of a claim could be found in the references, but it requires that the information combined would teach all aspects of an invention sought to be patented and render the invention obvious. In this case, the Examiner has picked out various pieces of information from the references cited, which combined, do

not teach the present invention. Moreover, Applicant argues, the relevance of some references is questionable.

Applicant notes that, as previously argued, Hodgson does not disclose or suggest that the fragments are ligated without first being purified or that each vector segment comprises a selectable and/or a counter-selectable marker. Applicant argues that Hodgson explicitly states that "the released insert fragments are isolated and purified from the vector fragments (paragraphs [0030] and [0051]). In yet another preferred embodiment, the insert DNA fragments are created from synthetic oligodeoxynucleotides (ODNs), by means of any methods for using either annealed, double-stranded ODNs, or DNA molecules made by polymerizing annealed ODN sequences to fill gaps. Synthetic ODNs can be ordered from a commercial supplier (e.g., MWG, Inc., Charlotte, N.C.), and should be purified (paragraph [0040]). Applicant argues that it is a well-settled law that an "omission of an element and retention of its function is an indicia of unobviousness." In re Edge, 359 F2d 892, 149 USPQ 556 (CCPA 1966)." MPEP § 2144.04.II.B.

Applicant argues that a fundamental aspect of the invention is the coordinated use of structurally distinct vectors comprising selectable and/or counter selectable markers, which is not taught by Hodgson and Padgett et al. Hodgson discloses that the colonies are selected using blue-white selection indicative of functional β-gal expression or absence thereof. This simplified clone selection method is most suitable, if not singularly suitable, for methods entailing the presence of only one vector. Notably, the blue-white selection method does not allow differentiation between the clones having

the desired construct and the clones having a single insert ligated back into the vector it was excised from. Therefore, in a sense, Hodgson teaches away from the subject matter of the independent claims 1, 2, and 35. In addition, since Hodgson purifies his fragments of interest, one of skill in the art would have figured out that counter selection markers are not needed in Hodgson's invention.

As provided in the Declaration of Dr. Reisinger submitted in response to the previous office action, the use of two-marker vectors provided unexpected and clear advantage over one-marker vectors. Specifically, when the inventors used one-marker vectors, they found that 28% of the clones were false-positives. Such a high percentage of false positive clones necessitates additional steps of verification whether the selected clone is truly or falsely positive. In contrast, when two-marker vectors were used, no false positive clones were encountered. Accordingly, the use of two-marker vectors, as claimed by the Applicant, makes the multiple-fragment cloning procedure much more efficient. Hodgson admits that cloning using Type IIS enzymes is a paradigm of trial and error, and that more work needs to be done before cloning multi-segment DNA molecules. (paragraph [0009]). It follows that at the time the instant invention was made, there was no reasonable expectation of success in cloning multiple pieces of DNA into one vector through a one-step process.

With respect to Padgett et al., Applicant argues that the reference does not mention, let alone "teach," that ligating digested fragments does not need purifying them. Applicant argues that Padgett et al. is irrelevant to the present invention because the increased efficiency required the use of a methyl-sensitive Type IIS restriction

enzyme and methylated CTP, which is inapposite to the present invention. Including the methylated CTP in the PCR reaction acted to inhibit digestion of Eam1104I sites within the amplicon, and thus resulted in increased cloning efficiencies by ensuring that only the non- methylated, Eam1104I site on the 5' primer, and not the Eam1104I site within the amplicon, was competent for digestion. Quite simply, no increase in cloning efficiency would have been observed by Padgett et al. without the use of a methyl-sensitive Type IIs restriction enzyme and methylated CTP. Moreover, Applicant argues, Padgett only teaches simple cloning of PCR fragments with a single vector, which does not approach the complexity of cloning 3 or 4 vectors simultaneously as with the present invention. As for page 35, column 1 of Padgett et al., Applicant speculates that the Examiner considered the sentence "[c]oncurrent digestion of insert and target PCR products and a brief ligation step of the digested material in the presence of the ENase further reduce the overall cloning time" as teaching "ligating digested fragments without purifying them." However, Applicant argues, given that Padgett et al. do not teach "ligating digested fragments without purifying them" in the experimental or discussion section, the mere mentioning of "[c]oncurrent digestion of insert and target PCR products and a brief ligation step of the digested mater" would add little, if anything, to the teaching. The mere language would not teach the concept of "ligating digested fragments without purifying them," because between the step of "concurrent digestion of insertion and target PCR products" and the "a brief ligation step" may well include a purification step. Applicant argues that the fact that certain step is not mentioned does not necessarily indicate that this step is omitted. Thus, to interpret the language to

mean that the step of "digestion of insert and target PCR products" and the step of "ligation" are "concurrent" would be not only implausible but also contrary to experimental conventions. Even assuming that Padgett et al. do not involve an isolation or purification step, the reference would still not be applicable to the present case. In the present invention, vectors contain inserts and upon digesting vectors and re-ligation of inserts, unnecessary vectors are discarded. Thus, the ratio of inserts to the respective vectors is 1:1. Whereas in Padgett et al., inserts are amplified by means of PCR. Thus, in the digestion (and ligation) mixtures, the vectors and the respective inserts are present in a different ratio. Assuming that Padgett performed 25 cycles of PCR with 100% efficiency of PCR amplification, the ratio of inserts to vectors would be about 16,000,000 (225) to 1, and assuming a 40% efficiency, the ratio of inserts to vectors would be about 6000 to 1. Even assuming a 20% efficiency of PCR amplification, the ratio of inserts to their respective vectors would still be more than 100 to 1. Thus, for all intents and purposes, in a practical sense, the PCR mix of Padgett et al. has been purified. Thus, without explicitly stating so, Padgett et al. do teach purification of the insert by enriching the ratio of insert to vector through PCR.

Applicant argues that, if one considers the teachings of Hodgson and Padgett as a whole, these teachings may be reconciled as follows: if one uses PCR to enrich the relative amount of the inserts or if the inserts are provided as vectorless oligonucleotides, then purification may be unnecessary (according to Padgett) but if the inserts are excised from their respective vectors and not enriched (by PCR or otherwise), then they must be purified (according to Hodgson). Notably, the

experimental mixture recited in the claim is more similar to the experimental design described in Hodgson than to Padgett. Accordingly, the teaching of Hodgson (purification) is more relevant than the teaching of Padgett (no purification if insert amounts are enriched). Moreover, Applicant argues, Hodgson was filed after Padgett et al. was published. Thus, Padgett et al. do not challenge the disclosure that "the construction of complex DNA molecules by this approach was limited to a small number of examples." Further, it may be said that the state of the art was more advanced at the time of Hodgson publication, compared to the state of the art at the time of Padgett publication. And yet, at the later time, the state of the art leads to a conclusion that the construction of complex DNA molecules is generally unsuccessful. Accordingly, the reasonable expectation of success is still lacking, even if the Examiner's assertion were fully accredited. Therefore, Padgett et al. do not remedy Hodgson in respect of a skipping isolation/purification step, as the Examiner had attempted to show.

With respect to Resnick et al., Applicant argues that the reference is not applicable to the present case due to the different contexts presented by the two cases. Resnick et al. is directed to a method of making a yeast artificial chromosome (YAC) comprising introducing into a yeast cells a population of nucleic acids and a vector, wherein the vector comprises a yeast centromere, a selectable marker, a yeast telomere, and a sequence which can recombine with a region of a nucleic acid within the population of nucleic acids, whereby in vivo recombination makes the YAC. The selectable marker or counter selectable marker is used in different contexts in Resnick et al. than in the instant application. First, Resnick et al. emphasized that the

selectable marker and counter selectable markers are used in a method of introducing a population of nucleic acids into yeast cells; second the method is used to create the YAC *in vivo*. In contrast, the present invention involves use of selectable marker and counter selectable markers to prepare precise genes encoding PKS modules, which are entirely done *in vitro*, and the product is used entirely for synthetic purposes. More significantly, Resnick et al. do not teach use of selectable marker and counter selectable marker in the same fashion as in the present invention. Applicants respectfully submit that the Examiner's assertion does not constitute a faithful reading of the reference. Simply because a reference mentions use of a selectable marker or counter selectable marker, it does not necessarily lead to the conclusion that any future use of the same would be obvious regardless of the contexts in which the selectable marker or counter selectable marker is used. Although as Resnick et al. stated, "selectable marker" and "counter selectable marker" are two terms familiar to one of ordinary skill in the art, use of them in a different context may not be feasible, or if feasible, may not be beneficial. In other words, whether the selectable marker and/or counter selectable marker would work as intended, i.e., the outcome of such uses, is not obvious. For one, Hodgson did not use them. Moreover, a closer look at Resnick et al. would reveal that this reference is non-enabling when applied to the present invention, because the method it teaches relies solely on the use of homologous recombination to create the large YAC sequence, not a recombinant, single-tube cloning method as with the present invention. In fact, Resnick et al. teach away from the present invention by stating that the use of recombinant cloning methods, such as

the one with the present invention, is problematic (see page 2, lines 28-30), which is what motivated them to use homologous recombinant methods instead.

With respect to Gokhale et al. Applicant argues that, as previously discussed, Gokhale et al. describe recombining modules from naturally occurring PKSs using conventional recombinant techniques. Gokhale et al. showed that modules that are not naturally found in the same polypeptide can be combined to produce catalytically functional products. Gokhale et al. does not have any bearing on the concurrent, directional cloning of multiple synthons as claimed in this application, given that none of the independent claims of the instant application, namely, claims 1, 2, 14, and 35, recite a specific gene such as recombinant PKS. In any event, if Gokhale et al. were relevant at all to the instant application, it would only be because it mentioned the PKS module, which is only remotely related to claims 11 and 12 of this application. Accordingly, Gokhale et al. is irrelevant to the analysis of obviousness of claims 1, 2, 14, and 35. Moreover, Gokhale et al. does provide support for the fundamental need in the art at that time for cloning large DNA segments, including, but not limited to, PKSs, as evidenced by the large number of DNA manipulations outlined in the methods that were required to actually clone the PKSs tested. The present invention answers the call for the need. However, Gokhale et al. simply does not provide motivation for one to combine Hodgson and other references cited; nor would the combination of them teach the present invention.

Applicant argues that paragraphs [0231]-[0235], the Figures, and the remainder of the specification illustrates that Type 1, Type 2 and Type 3 vectors have other

differences, not limited to the position of the selectable marker with relation to other vector elements and that none of these differences are found in the cited references.

Claims 3-13 are directed to a method for ligation of a plurality of DNA segments, directly or indirectly dependent from claim 2; claims 15-19 are directed to compositions of an unpurified cleavage products of DNA molecules and a DNA ligase, directly or indirectly dependent from claim 14; claims 31-34 are directed to a method of ligation of a plurality of DNA segments, directly or indirectly dependent from claim 1; and claims 36-39 are directed to a method for ligation of a plurality of DNA segments, directly or indirectly dependent from claim 35. The Examiner rejected these claims in sweeping language without elaborating how all of the limitations of these claims are taught by the references cited.

As discussed above, the references cited by the Examiner, when combined, do not render independent claim 1, 2, 14, or 35 obvious; therefore, neither are dependent claims 3-13, 15-19, 31-34, and 36-39 obvious over Hodgson, in view of Padgett et al., Resnick et al., and Gokhale et al.

Moreover, Applicant argues, the cited references do not teach, but not limited to, the following features of the present invention:

(1) in claim 3: "selecting transformants that express said first and second selectable markers and do not express said first, second, or third counter-selectable marker";

(2) in claim 5: "said first and second selectable markers are genes conferring drug resistance";

(3) in claim 6: "said first, second and third counter-selectable markers are selected from

the group consisting of ccB (anti-DNA gyrase protein), sacB (sucrose sensitivity), araB (ribulose sensitivity), and tetAR (tetracycline resistance/fusaric acid hypersensitivity";

(4) in claim 7: "a) said first and third cleavage sites are the same; b) said second and fourth cleavage sites are the same; c) the 5-prime cleavage site of at least one Type 3 DNA molecule is the same as the 3-prime cleavage site of the same Type 3 DNA molecule; and/or d) the 5-prime cleavage site of at least one Type 3 DNA molecule is the same as the 5-prime cleavage site of a different Type 3 DNA molecule";

(5) in claim 11: "the DNA segments of the Type 1, Type 2 and Type 3 DNA molecules comprise sequences encoding a polypeptide segment of a polyketide synthase";

(6) in claim 12: "the DNA segments of the Type 1, Type 2 and Type 3 DNA molecules comprise sequences encoding a polyketide synthase domain";

(7) in claim 13: "the DNA molecules cleaved in step (b) are cleaved in the same container";

(8) in claim 15: The composition of claim 14 "comprising at least two Type 3 DNA molecules";

(9) in claim 16: The composition of claim 14 "comprising an endonuclease that cleaves at the first, second, third, or fourth cleavage sites or at one or more 5-prime or 3-prime cleavage sites";

(10) in claim 18: The composition of claim 16 that "contains at least two Type 3 DNA molecules comprising 5-prime or 3-prime cleavage sites and wherein the endonuclease cleaves at the third and fourth cleavage sites and at the 5-prime and 3-prime cleavage sites of said Type 3 DNA molecules";

(11) in claim 19: The composition of claim 14, wherein, "after a sufficient amount of time for a ligation reaction using said DNA ligase, a product is formed, the product comprising: the first DNA segment immediately upstream of the second DNA segment, wherein the second DNA segment is immediately upstream of the DNA segment from at least one Type 3 DNA molecule; and at least one selectable marker";

(12) in claim 31: "the selectable markers in (a) are sequences encoding a protein that confers drug resistance to a host, and the selection in step (d) is based on the presence in a vector comprising the ligation product of two different selectable markers, wherein the two different selectable markers are associated with two different vectors in step (a)";

(13) in claim 32: "the selectable marker provides resistance to a drug selected from the group consisting of carbenicillin, tetracycline, kanamycin, streptomycin, and chloramphenicol";

(14) in claim 33: "the selection in step (d) is based on the absence in a vector comprising the ligation product of a counter-selectable marker present in one or more of the vectors in (a)";

(15) in claim 36: "said selecting is based on the presence in the ligation product of both the first and second selectable markers";

(16) in claim 37: "the selectable markers provide resistance to a drug selected from the group consisting of carbenicillin, tetracycline, kanamycin, streptomycin, and chloramphenicol"; and

(17) in claim 39: "at least one of the selectable markers is a counter selectable marker

selected from the group consisting of ccdB (anti-DNA gyrase protein), sacB (sucrose sensitivity), araB (ribulose sensitivity), and tetAR (tetracycline resistance/fusaric acid hypersensitivity)."

Therefore, Applicant requests the withdrawal of the rejection.

Applicant's arguments are acknowledged, however the rejection is maintained for the following reasons:

In response to Applicant's argument that the Examiner's conclusion of obviousness is based upon improper hindsight reasoning, it must be recognized that any judgment on obviousness is in a sense necessarily a reconstruction based upon hindsight reasoning. But so long as it takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made, and does not include knowledge gleaned only from the Applicant's disclosure, such a reconstruction is proper. See *In re McLaughlin*, 443 F.2d 1392, 170 USPQ 209 (CCPA 1971).

In response to Applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.,* 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). None of the references has to teach each and every claim limitation; if they did, this would have been anticipation and not an obviousness-type rejection. Therefore, Applicant's arguments are not found persuasive because Applicant did not address the

combination of references. Furthermore, as per Applicant's admission, most of his arguments are not new; these arguments were previously addressed. Therefore, only the new arguments are addressed below.

Applicant argues that Hodgson's teachings in paragraph 0009 indicate that there was no reasonable expectation of success in cloning multiple pieces of DNA into one vector through a one-step process. This is incorrect. Paragraph 0009 only refers to data obtained before Hodgson's invention. Hodgson teaches his invention as solving the problem of constructing complex DNA molecules such as genes and chromosomes by cloning multiple pieces of DNA into one vector (see paragraphs 0014-0022, 0030, 0031-0033). Therefore, one of skill in the art would have reasonably expected to be successful in assembling complex DNA molecules.

Applicant argues that Padgett et al. is irrelevant to the present invention because the increased efficiency required the use of a methyl-sensitive Type IIS restriction enzyme and methylated CTP, which is inapposite to the present invention. This is not found persuasive. Padgett et al. teach that their Type IIS restriction enzyme is inhibited by the methylation in the recognition sequences; they teach that, when using PCR to obtain the insert to be ligated and cloned, methylated CTP must be included in the PCR reaction in order to methylate the recognition sequences and avoid degradation of the insert itself. Of course no increase in cloning efficiency would be observed without the use methylated CTP because the inserts would have been digested. This does not mean that their teachings do not apply to the instant invention. The claims encompass using any Type IIS enzyme, including using methylation-sensitive enzymes (in which

case, Applicant would have to use methylated CTP to avoid digestion of the fragments to be ligated). Furthermore, Padgett et al. clearly teach that the PCR products were digested with the Type IIS restriction enzyme and ligated in the presence of this Type IIS restriction enzyme (i.e., without purification) and that it is the presence of the Type IIS restriction enzyme in the ligation mixture which provides a selection for the formation of the desired ligation product; methylated CTP has nothing to do with this selection (p. 34, column 1). By reading Padgett et al. one of skill in the art would have known that their teachings of ligating without purifying the digest applies to any Type IIS enzyme. Moreover, as Applicant is well aware, "concurrent" means occurring simultaneously (i.e., without any intervening step). Based on all of the above, it is not clear why Applicant argues that the teachings of Padgett et al. imply purification. The reference is very clear: there is no purification. The rest of Applicant's arguments regarding Padgett et al. are not found persuasive because they are individually directed to Padgett et al. and it does not address the combination of references on which the rejection is based.

Applicant argues that, if one considers the teachings of Hodgson and Padgett as a whole, these teachings may be reconciled as follows: if one uses PCR to enrich the relative amount of the inserts or if the inserts are provided as vectorless oligonucleotides, then purification may be unnecessary (according to Padgett) but if the inserts are excised from their respective vectors and not enriched (by PCR or otherwise), then they must be purified (according to Hodgson). This is just an assumption not supported by any evidence. Applicant did not provide any evidence

indicating that the method of Padgett et al. with its advantages cannot be applied to Hodgson's method. "May be reconciled" does not equal evidence.

Applicant argues that the teachings of Resnick et al. are not applicable to the present case due to the different contexts presented by the two cases, i.e., they solely rely on the use of homologous recombination to create the large YAC sequence, not a recombinant, single-tube cloning method as presently claimed. Applicant also argues that Resnick et al. teach away from the present invention. These arguments are not found persuasive. The claimed invention is already taught by Hodgson and Padgett et al. Resnick et al. was only cited for teaching that selectable and counter-selectable markers were routinely used in the prior art. Applicant did not provide any evidence that it would not have been within the capabilities of one of skill in the art to use such markers in any cloning method. In fact, in his remarks filed on 05/13/2008, Applicant admits that selectable and counter-selectable markers have been known in the art for decades and those of skill have known for decades how to use such markers to select ligation products.

Applicant argues that, by purifying the fragments, Hodgson teaches away from the instant claims and that one of skill in the art would have figured out that counter selection markers are not needed in Hodgson's invention. This is not found persuasive. Again, the rejection is based on all the combination of all the cited references which teaches that the fragments are not purified and that selectable and counter-selectable markers are used.

Applicant argues that claims 3-13, 15-19, 31-34, and 36-39 were rejected in sweeping language without elaborating how all of the limitations of these claims are taught by the references cited. Applicant also argues that the references do not teach all claim limitations. This is incorrect. All claims limitations have been addressed in the rejection above. The only limitation which was not specifically named was cleaving in the same container (claim 13). However, such is implicit to the method taught by the combination of art above.

Applicant is right in noting that the citation of Steward et al. was not provided. This was an inadvertent error on Examiner's part. Both Resnick et al. and Steward et al. were cited for teaching selectable and counter-selectable markers. Citing Steward et al. is therefore redundant; rejecting the claims does not require this reference.

With respect to the advantage of eliminating isolating and recloning of small polynucleotide fragments, this is already taught by the prior art (see the rejection above).

For the reasons set forth above, Applicant's arguments are not found persuasive and the rejection is maintained.

Conclusion

4. **THIS ACTION IS MADE FINAL.** Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within

TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ILEANA POPA whose telephone number is (571)272-5546. The examiner can normally be reached on 9:00 am-5:30 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joseph Woitach can be reached on 571-272-0739. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Ileana Popa/
Primary Examiner, Art Unit 1633